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3. TRANSPORT OF DANGEROUS BIOLOGICAL MATERIALS

3.1. Introduction

Transport of biological samples is an important stage for successful laboratory identification and confirmation of bio-threats in field or reachback facilities. Transport of biological samples should be carried out properly to assure security and safety for humans, environment and specimen. Moreover package with infectious substances must be delivered safely, efficiently and timely to the place of destination to reduce content degradation. Hence, shippers must prepare package containing sample in way that minimize the risk of damage during transport and uncontrolled release of dangerous material into the environment. Samples must also be protected from contamination by the appropriate vessels or jars. Type of sample may determine the need to use refrigerants such as dry ice or coolers. Depending on potential and real content of infectious biological materials in specimen, it may be considered as dangerous goods or dual-use material, which falls under legal restrictions. Due to that, it is very important for shippers to be familiar with the current requirements concerning transport of infectious materials. They should be trained frequently to make sure that they will handle with the package confidently and will classify the material properly.

3.2. Dangerous goods and dual-use materials regulations

The biological infectious materials belong to dangerous goods, which may present hazards for human and environment during the transport. Transport of dangerous good is an area of recommendations of United Nations. These recommendations are a base for international, national and internal regulations for example for roads, maritime, railways or air. These regulations describe dangerous goods in 9 classes, including biological ones (subclass 6.2 – biohazard). For purposes of transport, Infectious substances are defined as substances which are known or are reasonably expected to contain pathogens. Pathogens are defined as microorganisms (including bacteria, viruses, rickettsiae, parasites,

fungi) and other agents such as prions, which can cause disease in humans or animals. There are several types of goods in this subclass: biological substances Cat. A (UN 2814) and Cat. B (UN 3373), bio-medical and clinical wastes (UN 3291), infectious substances affecting animals only (UN 2900). Moreover, the conditions of transport of hazardous substances or technical requirements concerning packages and their testing are described. WHO periodically issues comprehensive guideline solely on transport of infectious substances, which is very helpful tool for shippers of biological materials. This chapter bases mainly on this publication.

Furthermore, some biological substances are considered as dual-use materials due to biological weapons proliferation threat. A certain species of viruses, bacteria or toxins which may be pathogenic for human, animal or cultivation – they additionally represent a threat for national or international security. That is why, there might be some national or international restrictions in possession, transfer, export or import of these materials. There is a regulation in European Union, which covers dual-use biological substances. It establishes control measures for their export and transits, thus knowledge about it and other national regulations is important for successful transport of selected biological materials. The informal international forum of states – the Australian Group – is aimed at harmonising export controls in the field of biological and chemical dual-use materials and enhance implementation of the Biological and Toxin Weapons Convention and the Chemical Weapons Convention. Its unofficial publication control list handbook is a helpful tool for shippers of biological materials (Australia Group Common Control List Handbook Volume II: Biological Weapons-Related Common Control Lists, Revision 2, January 2016).

3.3. Transport of infectious samples

Laboratory analysis cannot be done right if the material has been collected or transported incorrectly or stopped during transport. Hence, the organization of shipment plays important role in timely, safe and secured transport. Shippers are mostly responsible for planning, activity during the preparatory phase, sample delivery and all the associated aspects. Shippers should ensure that packages are prepared in a proper way and that they won't be damaged during transport or pose any threat to surrounding (people or animals). The shipper discusses and arranges with the courier the type of packaging, conditions and means of transport (most direct and shortest is preferable), and also obtains potential export permission. Their role is also to prepare the documentation. Shipment has to be supplemented with the appropriate consignment note. Depending on internal procedures and current legislation, it typically contains: information of a person who prepared the package, sender, recipient responsible person for shipment

(with telephone number), packing list (a short description of the sample such as the kind of material (liquid, solid, gas substance), quantity, volume, weight of the material, number of vials, real or potential name of biological agents), assignment to appropriate hazard class connected with proper labelling and marking on the outer package. Packages from sampling mission often contain more information such as: place of sampling (its description, grid coordinates, images), date and time of sampling, sample identification number, results of rapid tests which were done on site as well as sample collection method, type of preservation and analysis requested by shipper. Depending on the content and way of transport package may contain the shipper's *Declaration of Dangerous Goods*, import/export permits, an air waybill for air transport or equivalent documents for road, rail and sea shipments or other document required by courier. In case of international transport, for customs purposes a pro-forma invoice is also mandatory, which contains the sender and recipient's address, the number of packages, details of content and purpose, weight, commercial value (for diagnostic specimens *No commercial value* should be written). Before shipment sender has to ask recipient about the readiness to accept the shipment in anticipated time.

Furthermore, the shipper has to choose the right means of transport adjusted to a specific sample and to secure the shipment against access by unauthorized persons. In military, alleged use of biological warfare and criminal investigation contexts additionally, a chain of custody is observed. It is the process of tracing a sample from the place of collection to the laboratory until the analysis. Each stage of transfer and receipt of the sample must be appropriately documented, to assure and maintain the sample integrity. This is done using the appropriate *Chain of Custody (COC)* documentation. The form must be filled out by all persons who take part in transport. A chain of custody is initiated by the sampler whose task is to properly collect and mark samples. During the transport, each person receiving and transmitting the sample is required to complete the form. The basic information contained in the form may include: short description of shipment (codes number), information about shipper and recipient – addresses and phone numbers, special handling requirements, printed name and signature of person relinquishing custody (date and time when custody was relinquished), printed name and signature of person receiving custody (date and time receipt of the sample). An example COC is shown below.

On the other hand courier role is to advise the shipper about current regulations and packages selection, archiving documentation and taking a part in chain-of-custody if applicable. The role of recipient is to confirm readiness to receive shipment, organize timely package collection and if applicable, obtain import permission and organize custom procedures. Finally recipient confirms sender about successful shipment arrival, including information about quantity and quality of content.

Sender and contact name			Recipient and contact name	
Ad- dress			Address	
Phone			Phone	
Sample ID	Data and time	Sample description	Type of sample	Special instructions/ comments:
Chain of custody				
Received from		Released by:	Received from:	Received by:
Signature:		Signature:	Signature:	Signature:
Date/time:		Date/time:	Date/time:	Date/time:
Received from:		Received from:	Received from:	Received by:
Signature:		Signature:	Signature:	Signature:
Date/time:		Date/time:	Date/time:	Date/time:

Figure 1. Example chain-of-custody form (COC)

3.4. Types of infectious material categories and packages

The dangerous goods shipment, has to be marked and identifiable by UN numbers and shipping names, according to their classification and composition. The main division of infectious shipments (Subclass 6.2) falls into two categories: Category A (UN 2814 or UN 2900), and Category B (UN 3373).

Category A infectious substances are defined as any material that contains or is reasonably expected to contain a pathogen transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. An exposure occurs when substance released from its protective packaging contact with humans or animals. If so, it has to be marked as UN 2814 (INFECTIOUS SUBSTANCE, AFFECTING HUMANS). Infectious substances cat. A which cause disease in animals only should be assigned to UN 2900 (INFECTIOUS SUBSTANCE, AFFECTING ANIMALS). An substance which does not meet the criteria for category A (such as effects of exposures related with transport, severity and prognosis of potential disease) should be assigned to UN 3373 (BIOLOGICAL SUBSTANCE, CATEGORY B).

Assignment to any of this category should be based on the known medical history and symptoms, form of transport (culture or clinical or environmental samples), endemic conditions and professional judgment. The WHO Guidance

includes an indicative (not exhaustive) list which helps in final categorization of biological material. Examples of infectious substances classified into category A are listed in Tab. 1.

Table 1. Indicative list of examples of infectious substances category A (adapted from the 18th edition of the United Nations Model Regulations).

UN number and shipping name		Microorganism
UN 2814 Infectious Substances, affecting humans	Viruses shipped in any form	Variola virus; Monkey pox virus; Ebola virus, Marburg virus; Crimean- Congo haemorrhagic fever virus; Lassa virus; Nipah virus; Omsk haemorrhagic fever virus; Junin virus; Kyasanur Forest disease virus; Machupo virus; Sabia virus; Guanarito virus; Hantaan virus; Hantaviruses causing haemorrhagic fever with renal syndrome; Hendra virus; Flexal virus
	Viruses shipped in form of culture	Yellow fever virus; West Nile virus; Dengue virus; Japanese encephalitis virus; Venezuelan equine encephalitis virus; Eastern equine encephalitis virus; Russian spring-summer encephalitis virus; Tick-borne encephalitis virus; Hepatitis B virus; Herpes B virus; Human immunodeficiency virus; Highly pathogenic avian influenza virus; Poliovirus; Rabies virus;
	Bacteria shipped in form of culture	<i>Yersinia pestis</i> , <i>Bacillus anthracis</i> ; <i>Francisella tularensis</i> ; <i>Clostridium botulinum</i> ; <i>Brucella abortus</i> ; <i>Brucella suis</i> ; <i>Brucella melitensis</i> ; <i>Coxiella burnetii</i> ; <i>Burkholderia mallei</i> - <i>Pseudomonas mallei</i> ; <i>Burkholderia pseudomallei</i> - <i>Pseudomona pseudomallei</i> <i>Escherichia coli</i> (verotoxigenic); <i>Chlamydia psittaci</i> (avian strains); <i>Mycobacterium tuberculosis</i> ; <i>Coccidioides immitis</i> ; <i>Rickettsia prowazekii</i> ; <i>Rickettsia rickettsia</i> ; <i>Shigella dysenteriae</i> type 1
UN 2900 Infectious substances, affecting animals only	Viruses shipped in form of culture	African swine fever virus; Classical swine fever virus; Foot and mouth disease virus; Avian paramyxovirus Type 1-Velogenic Newcastle disease virus; Lumpy skin disease virus; Peste des petits ruminants virus; Rinderpest virus; Sheep-pox virus; Goatpox virus; Swine vesicular disease virus; Vesicular stomatitis virus

There are some biological materials which are *NOT* subject of dangerous goods regulations:

- Substances containing microorganisms that are non-pathogenic to humans or animals;
- Substances with neutralized or inactivated pathogens;
- Environmental samples (including food and water samples) not posing a significant risk of infection;
- Some clinical materials, such as: dried blood spots (collected by applying a drop of blood onto absorbent material), faecal occult blood screening tests, blood or blood components (for the purposes of transfusion or for the preparation of blood products), tissues or organs (for transplantation).

Human or animal specimens (patient specimens) for which there is very little likelihood of pathogens presence (e.g. transported for routine testing not related to the diagnosis of an infectious disease), are also exempted from Subclass 6.2 under the following conditions: triple leak-proof packaging marked as Exempt human or animal specimen. The likelihood assessment has to be based on professional and comprehensive judgement.

Medical or clinical wastes containing infectious substances category A should be shipped under the name UN 2814 or UN 2900, as appropriate. Wastes containing substances category B should be shipped under the name UN 3291 - CLINICAL WASTE, UNSPECIFIED, N.O.S. or (BIO) MEDICAL WASTE, N.O.S. or REGULATED MEDICAL WASTE, N.O.S.

The general triple packaging system consists of three layers as follows:

- Primary receptacle – a watertight, leak-proof or stiff-proof receptacle containing the specimen;
- Secondary packaging – resistant, watertight, leak-proof or stiff-proof packaging to enclose and protect the primary receptacle(s);
- Outer packaging – shipping packaging with suitable cushioning material protecting their contents from physical damage during transport.

Overall external dimension should be at least 10 x 10 cm. In case of liquids, there should be an absorbent material placed between the primary receptacle(s) and the secondary packaging to absorb the entire contents released during transport. Several cushioned primary receptacles may be placed in one secondary packaging, but with the use of sufficient additional absorbent material; if they are fragile, they should be either individually wrapped or separated to prevent contact between them. Each completed package has to be correctly marked, labelled and accompanied with appropriate shipping documents (as applicable).

In case of substances that must be transported refrigerated or frozen, appropriate temperature label may be provided. A refrigerant (e.g. ice, dry ice) should be placed around the secondary packaging, which position has to be secured within the outer package (in case of melting of refrigerant). The primary

receptacle and the secondary packaging shall keep its integrity at the lower temperatures e.g. samples transported in liquid nitrogen should be placed in plastic primary receptacles, capable of withstanding very low temperature. In case of dry ice the outer packaging has to allow the release of carbon dioxide gas. In case of using ice, the outer packaging should be leak-proof. In case of usage of dangerous refrigerant (e.g. solid carbon dioxide, cryogenic liquids) appropriate information about it shall be included on outer package. Moreover in case of use solid carbon dioxide additional packaging instruction has to be followed (PI003 or PI945). The outer package need to be additionally marked with label (Fig. 2) number UN 1845, and information *CARBON DIOXIDE, SOLID, AS COOLANT* with the net quantity of dry ice in kilograms.

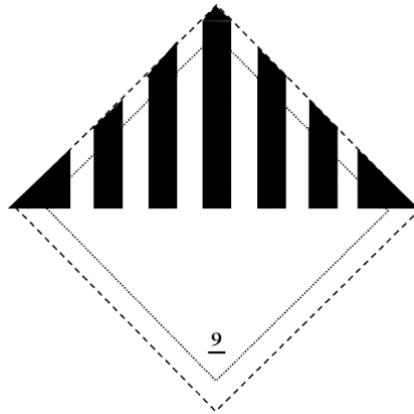


Figure 2. Hazard label for miscellaneous dangerous substances e.g. for solid carbon dioxide, solid (dry ice) (UN 1845) (Image adapted from WHO Guidance on regulations for the transport of infectious substances 2015–2016, WHO/HSE/GCR/2015.2, World Health Organization, Geneva, 2015)

Several of the same type of packaging can be combined together to form one unit – an ‘overpack’, which is then sent to the same destination. Overpacks can contain dry ice, if needed. In the case of using ice, the overpack should be leak-proof. All markings given on outer packages should be clearly placed (repeated) on overpacks. This information includes the shipper name, the telephone number of responsible person, the recipient’s name and address, the proper shipping name with the United Nations number.

Due to various hazard levels, detailed packing instructions are different for infectious substances Category A (UN 2814 and UN 2900) and for infectious substances category B (UN 3373). The main differences concerning types of packaging, labelling and documentation are discussed below.

3.4.1. Category B substances

The samples under UN 3373 are subjected to transport and packing instructions P650. The package for category B should consist of three components, as mentioned before. The package has to be strong enough to resist the shocks, temperature and humidity changes, vibrations, loadings normally encountered during transport. The primary or secondary package needs to resist differential pressure 95 kPa without leakage. For air transport the outer package need to be rigid. Typical packaging and labelling is shown in the figure below.

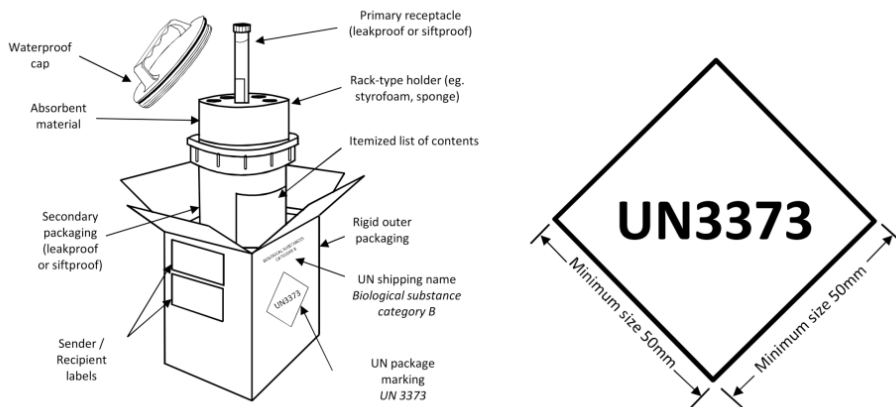


Figure 3. Example of package for category B infectious substances. Marking rhombus sign for category B infectious substances (Image adapted from WHO Guidance on regulations for the transport of infectious substances 2015–2016, WHO/HSE/GCR/2015.2, World Health Organization, Geneva, 2015)

There is no maximum quantity per package, in case of surface transport. Whereas in case of an air transport for liquid substances the primary receptacle should not exceed 1 litre and the outer packaging must not exceed 4 litres. For solid substances the outer packaging must not contain more than 4 kg (with exception to organs, body parts, whole bodies). The outer packaging should contain the following information:

- Sender's details (name, address, telephone number);
- Recipient's details (name, address, telephone number);
- Information that the package contains biological material – shipping name 'BIOLOGICAL SUBSTANCE, CATEGORY B' adjacent to the appropriate rhombus sign with the 'UN 3373' wording used for marking category B infectious substances (Fig. 3).

3.4.2. Category A substances

Category A substances (UN 2814 and UN 2900) have to be transported in triple packaging system produced and certified against special UN class 6.2 requirements. These include 9 m. drop test, a puncture test, a stacking test and internal pressure test of primary or secondary receptacle (without leakage in differential pressure not less than 95 kPa and temperatures in the range -40°C to $+55^{\circ}\text{C}$). Finally package need to be labelled by manufacturer with United Nations packaging specification marking. Packaging need to follow P620 instruction. Likewise category B, for transport by roads, rail or ship there are no limits regarding quantity or weight per package, but there are limitations of content for air transport: 50 ml or 50 g for passenger aircraft, 4 litres or 4 kg for cargo aircraft. Both primary receptacle and secondary packaging have to leak-proof, whereas outer package has to be rigid. If there is more than one of inner packaging, they should belong to subclass 6.2, other materials and dangerous goods are granted in limited amounts only for maintaining the viability, stabilizing or preventing degradation or neutralizing the hazards of the infectious substances. Substances that can be transported at room temperature or higher, should be placed in primary receptacle made of glass, metal or plastics with leak proof sealing. Typical packaging and labelling is shown in the figure below (Fig. 4).

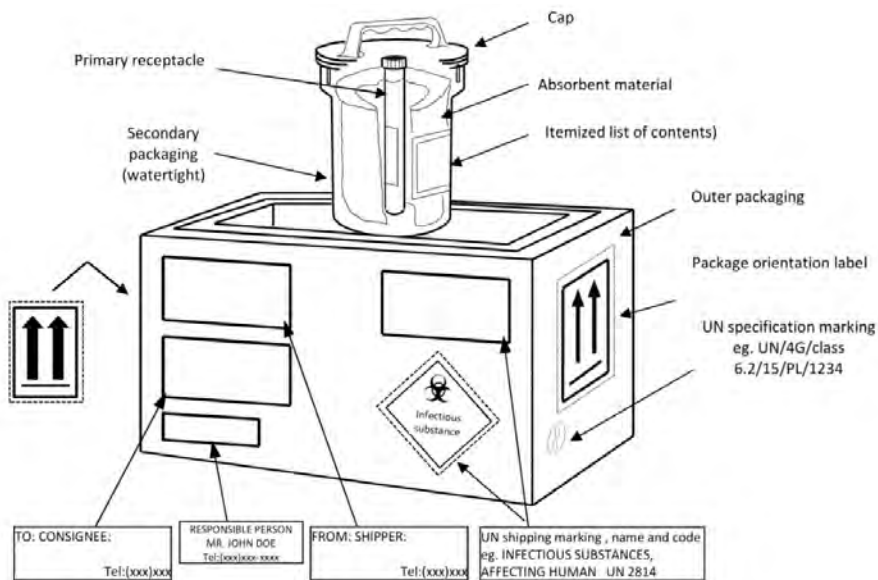


Figure 4. Example of triple packaging system for the packaging and labelling of Category A infectious substances (Image adapted from WHO Guidance on regulations for the transport of infectious substances 2015–2016, WHO/HSE/GCR/2015.2, World Health Organization, Geneva, 2015)

Between the secondary packaging and the outer packaging, a detailed list of the contents shall be enclosed. For air transportation, the shipper's Declaration of Dangerous Goods has to be attached, also indicating other than 6.2 subclass of dangerous goods e.g. solid carbon dioxide. If the transported substance is unknown, but there is a reasonable suspicion that meets the criteria for inclusion in category A, it should be indicated with the words Suspected category A infectious substance (in documentation but not on the package).

The outer packaging should contain the following information:

- sender details (name, address, telephone number);
- telephone number of the person responsible for shipment;
- recipient's details (name, address, telephone number);
- information that the package contains Category A biological material
- shipping name: *UN 2814 INFECTIOUS SUBSTANCE, AFFECTING HUMANS* or *UN 2900 INFECTIOUS SUBSTANCE, AFFECTING ANIMALS ONLY*, adjacent with the appropriate rhombus with international biohazard sign used for marking category A infectious substances;
- Additionally for air transportation of volumes over 50 ml, orientation label (double arrows) has to be placed to indicate position of primary receptacles (Fig. 5).



Figure 5. Safety marks for Category A infectious substances (10 cm x 10 cm or 5 cm x 5 cm) and orientation arrows indicate position of primary receptacles (size A7) (Image adapted from WHO Guidance on regulations for the transport of infectious substances 2015–2016, WHO/HSE/GCR/2015.2, World Health Organization, Geneva, 2015)

References

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